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The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 4(4): 69-72

www.thepharmajournal.com
Received: 15-04-2015
Accepted: 20-05-2015

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Exhaled Nitric Oxide (NO) Levels as a Marker of COPD Exacerbation

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Abstract
Nowadays scientists are looking for the new non-invasive markers to detect early stages of COPD exacerbation. The aim of our work was to compare the levels of NO in exhaled air of patients with stable COPD and in COPD patients with acute exacerbations. Also we evaluated the effect of continuous use of inhaled corticosteroids (ICS) on the exhaled NO level. The study included 61 patients with COPD (group 1 – 38 patients in the stable phase, group 2 – 23 patients with exacerbation; group 3 – 11 healthy volunteers). Lung function, concentration of NO in exhaled air and assessment with mMRC scale were conducted in all patients.
The analysis revealed that NO level in exhaled air during exacerbation of COPD was 24 [17-40] ppb, which is significantly higher in comparison with both patients with stable COPD (9,5 [7-12] ppb) and healthy people (12,0 [10,0-15,0] ppb) ($p < 0,001$). Therefore NO level in exhaled air determination can be used as one of the non-invasive markers of exacerbation in COPD for early start of enhanced therapy. Lung function, body mass index, height, weight and smoking history did not affect the concentration of NO in the exhaled air in patients with COPD either during exacerbation or remission. Also, we found no influence of prolonged therapy with ICS on NO levels in COPD patients, which can be due to the small size of the group.

Keywords: COPD, exhaled nitric oxide (NO), exacerbation.

1. Introduction
Today, chronic obstructive pulmonary disease (COPD) remains one of the global health issues. According to the World Health Organization (WHO), more than three million people die of the disease exacerbation throughout the world each year [4]. Exacerbation is a key problem of managing such patients as economic costs of treatment during flare-up periods become many times higher and frequent and persistent exacerbations considerably deteriorate quality of life of patients and cause early disability [4].
Currently, researchers are actively looking for new non-invasive indicators to detect exacerbation of COPD at early stages. Exhaled nitric oxide (NO) levels is one of those modern markers. However, there are limited data reported in literature about the use of NO for COPD diagnose.
Philip O'Reilly *et al.* (2007) in their studies have shown that NO levels in patients with acute COPD exacerbation are lower than those in patients with bronchial asthma (BA) but higher than in healthy volunteers. Accordingly, the authors have concluded that NO cannot be used as a reliable marker of early COPD exacerbations [7]. At the same time, other authors have obtained results suggesting that in patients with severe COPD NO levels during their exacerbation periods were significantly higher than during remission [2].
Several researchers have studied the seasonality effect on NO levels in COPD patients. It was found that NO levels were higher in a cold season, particularly, in autumn, possibly due to increased incidence of viral infections in that period of a year [2].
According to other researchers, NO levels in smoking patients with stable COPD are much lower than those in non-smokers. However, there is no difference between former smokers and non-smokers [3, 5, 8].
A number of researchers have demonstrated that unlike bronchodilators in those who suffer from bronchial asthma inhaled glucocorticosteroids (IGCSs) have no effect on NO levels in COPD patients [3]. At the same time, Z. Zietkowski *et al.* obtained an opposite result: according to their data, IGCS post-treatment NO levels were significantly lower in COPD patients [9].
Therefore, given quite contradictory evidence available to date the use of NO as a marker of COPD exacerbation as well as effects of IGCSs on NO levels still need investigating.

In this regard, we aimed to compare exhaled NO levels in COPD patients during remission and exacerbation periods and to assess the effect of continuous use of IGCSs on NO parameters.

2. Materials and methods

The study included 61 patients with verified COPD. COPD presence, severity and group as well as the pathological process phase were assessed in accordance with the criteria set out by the Ministry of Health of Ukraine in Order № 555 dated June 27, 2013 [6].

All subjects were assigned to 2 groups by disease stage. Group 1 included 38 remitting patients, and Group 2 included 23 subjects with exacerbation. All patients received a standard therapy according to their disease stages and groups. Control group 3 included 11 apparently healthy never-smoker volunteers with normal respiratory function (RF).

During the study of the main groups, subjects were assigned to subgroups according to therapy they were receiving. Subgroup 1a included remitting COPD patients on non-IGCS therapy, and subgroup 1b contained COPD patients who were receiving IGCSs. Subgroup 2a — exacerbation COPD patients not receiving IGCSs. Subgroup 2b — exacerbation COPD patients receiving IGCSs.

COPD diagnosis in all patients was verified by using RF parameters measured with Master Lab system (Jaeger, Germany): levels of forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1/FVC ratio were analysed; reversibility of airflow obstruction was tested with a short-acting β2-agonist (salbutamol). Obstruction severity was assessed by using the post-bronchodilator test (according to Recommendations of the Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2013).

Exhaled NO concentration was measured with Niox Mino system (Aerocrine, Sweden).

All patients were asked to complete the mMRC dyspnea scale.

Statistical analysis of the study data was performed with Statistika 6.1 software. Parametric statistics methods with calculation of mean values, standard deviation (M ± m) and the Student's t test were used for normally distributed variables. Non-parametric statistics methods with calculation of Med median and quartiles [25%–75%], Mann-Whitney and Kruskal-Wallis tests of significance were used for not normally distributed parameters. Findings were considered to be statistically significant at $p < 0.05$. Significance of differences by quality and binary parameters was assessed by using the chi-square ($\chi 2$) test where $n < 5$ by using the Fishers exact test for frequencies. Correlation analysis of exhaled NO level and RF parameters was also performed by using the Spearman's rank correlation coefficient (R).

3. Results and discussion

Groups of patients and subjects in the control group were comparable by age, sex, weight and BMI. Groups of COPD patients did not differ significantly by number of smokers, pack-year value or history of the disease. The number of exacerbations per year was significantly higher in patients who had flare-ups at the time of the study examination (Table 1).

Table 1: Comparison Group Description

Parameter	Group, n			P
	Group 1, n=38	Group 2, n=23	Group 3, n=11	
Gender, n (%)	m=32; (84.2) f=6; (15.8)	m=21; (91.3) f=2; (8.7)	m=10; (90.9) f=1; (9.01)	p_{1-2}=0.43; p_{1-3}=0.58; p_{2-3}=0.97;
Age, M±m, years	64.07±1.23	65.26±2.07	57.72 ± 2.87	p_{1-2}=0.51; p_{1-3}=1.0; p_{2-3}=1.0;
Weight, Med[25%-75%]	80 [72-86]	80 [60-89]	76[72-86]	p_{1-2}=0.56; p_{1-3}=0.9; p_{2-3}=0.65;
Smoking, n (%)	17 (44,7)	8 (34,8)	-	p_{1-2}=0.44;
BMI, kg/m^2 Med[25%-75%]	27.5 [23-30]	25 [23-29]	24 [22-28]	p_{1-2}=0.34; p_{1-3}=0.23; p_{2-3}=0.63;
Pack-year value, Med[25%-75%]	39 [22-60]	37 [15-45]	-	p_{1-2}=0.67;
Disease history, Med[25%-75%]	5 [3-9]	3,5 [2-10]	-	p_{1-2}=0.69;
Number of exacerbations per year Med[25%-75%]	1.0 [0-3,0]	2.0 [1.0-4.0]	-	p_{1-2}=0.00;

Assessment of lung function detected that FEV1 value was expectedly significantly lower than in patients from Group 1 and Group 2 versus healthy volunteers. And in patients with exacerbation FEV1 was also significantly lower than in remitting patients. At the same time, though FVC was significantly lower in patients from Groups 1 and 2 versus Group 3, the difference by this parameter was not significant between patients with exacerbation and those with stable COPD (Table 2).

Table 2: Study Group Description

Parameter	Group, n			P
	Group 1, n=38	Group 2, n=23	Group 3, n=11	
FEV1, Med[25%-75%]	51.5 [42.8-63.2]	40.1[32-52.6]	97.0 [90.0-100.0]	p_{1-2}=0.01; p_{1-3}=0.00; p_{2-3}=0.00;
FVC, Med[25%-75%]	87.2[81.8-91.4]	84.6[69.1-95.7]	102.0[98.0-110.0]	p_{1-2}=0.12; p_{1-3}=0.00; p_{2-3}=0.00;
FEV1/FVC, Med[25%-75%]	0.45[0.39-0.56]	0,38[0.32-0.57]	0,89[0.82-0.91]	p_{1-2}=0.23; p_{1-3}=0.00; p_{2-3}=0.00;

It also seemed appropriate to define the composition of the main comparison groups in accordance with the principles of the new COPD classification [1, 9]. It is found that there were patients of all categories in the stable patient group. Group C included the most number of patients, i.e. patients with minor clinical symptoms but severe obstruction and/or a history of exacerbations. In contrast to Group 1, Group 2 did not include any category a patients while Group D was the dominant one containing significantly more such patients versus the remitting patient group (Table 3).

Table 3: Study Subjects Distributed by COPD Groups

Classification Group	Group 1, n=38	Group 2, n=23	P
A, n (%)	5 (13.2%)	0	0.18
B, n (%)	9 (23.7%)	4 (17.4%)	0.56
C, n (%)	17 (44.7%)	7 (30.4%)	0.27
D, n (%)	7 (18.4%)	12 (52.2%)	0.00

Analysis of exhaled of NO levels helped determine that in Group 2 this parameter was significantly higher than in Group 1 (24 [17-40] ppb and 9.5 [7-12] ppb, respectively). In Group 3, this value was 12.0 [10,0-15,0] ppb and significantly ($p < 0.001$) lower than in Group 2. As for the remission period, NO levels in all patients in Group 1 was within the normal range and did not differ significantly from that of the control group. It is noteworthy, however, that minimum and maximum variance of the parameters was greater in Group 1 versus Group 3 (Fig. 1).

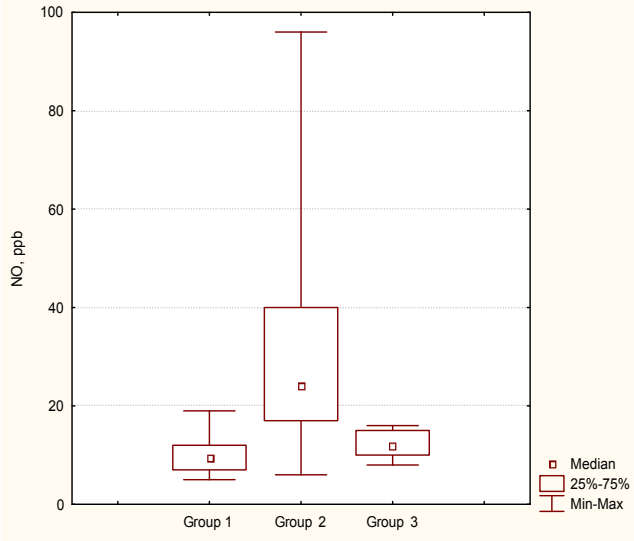

Fig 1: NO Values in Comparison Groups

Correlation analysis performed for COPD patients showed no correlation between exhaled NO levels and RF values in any of the comparison groups or in the general patient population (Table 4).

Table 4: RF/NO Correlation

Study Correlations	Group 1		Group 2	
	R	P	R	P
FEV1 and NO	0,27	0.1	-0.27	0.22
FVC and NO	0,17	0.29	-0.02	0.91
FEV1/FVC and NO	0,09	0.58	-0.23	0.33

We found no significant correlation between NO and height, weight and the pack/year value (Table 5).

Table 5: Anthropometric Indicators/Pack-Year Value to NO Levels Correlation

Study Correlations	Group 1		Group 2	
	R	P	P	R
Height and NO	-0.02	0.88	0.07	0.74
Weight and NO	-0.1	0.56	0.29	0.18
Pack-year value and NO	-0.27	0.1	0.22	0.32

No significant correlation between exhaled NO levels and the number of exacerbations a year ($R = 0.065$, $p = 0.7$ and $R = 0.046$, $p = 0.8$) was found in patients from Group 1 or Group 2.

Classification of patients by received treatment helped determine that NO did not differ significantly in patients treated or not treated with IGCSs for COPD exacerbation or during remission of the disease (Table 6).

Table 6: NO Levels/IGCSs Dependence

Parameter	Subgroup 1a	Subgroup 1b	Subgroup 2a	Subgroup 2b
Number of Patients, n (%)	20 (31.7%)	18 (28.6%)	10 (15.9%)	13 (23.8%)
NO, Med [25%-75%]	8,5 [7.0-12.5]	10,5 [7.0-12.0]	27,5 [15.0-40.0]	12,0 [17.0-29.0]

Notes:
$P_{1a-1b} - 0.81$;
$P_{1a-2a} - 0.001$;
$P_{1a-2b} - 0.00$;
$P_{1b-2a} - 0.001$;
$P_{1b-2b} - 0.001$;
$P_{2a-2b} - 0.95$.

4. Conclusions

1. In patients with exacerbation of COPD level of NO in exhaled air was 24 [17-40] ppb, which is significantly ($p < 0,001$) higher compared with healthy people (12,0 [10,0-15,0] ppb) and patients with disease in stable phase (9,5 [7-12] ppb).
2. Determining the level of NO in exhaled air can be used as one of the non-invasive markers of exacerbations in COPD for early start of more intensive therapy.
3. Lung function (FEV1, FVC, FEV1/FVC), BMI, height, weight and smoking history did not affect the concentration of NO in exhaled air in COPD patients either during exacerbation, or during remission.
4. Regular intake of inhaled corticosteroids had no influence on the level of exhaled NO in COPD patients, which may be due to the small size of the comparison group.

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